09/926323 09/926323

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FORM PTO-1390 (REV 5-93)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY DOCKET NO. 100564-00082
	TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371	DATE: October 15, 2001
		U.S. APPLN. NO. (IF KNOWN, SEE 37 C.F.R. 1.5) New 09/926323

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/03347	13 April 2000	13 April 1999

TITLE OF INVENTION: DIAGNOSTIC AND THERAPEUTIC USE OF ANTIBODIES AGAINST THE UROKINASE RECEPTOR

APPLICANT(S) FOR DO/EO/US: Manfred SCHMITT; Frank NOACK; Viktor MAGDOLEN; Henner GRAEFF; Thomas LUTHER; Sybille ALBRECHT; Martin MÜLLER; Olaf WILHELM and Nadia HARBECK

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371. (THE BASIC FILING FEE IS ATTACHED)

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This express request to begin national examination procedures [35 U.S.C. 371(f)] at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper demand for International Preliminary Amendment was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed [35 U.S.C. 371(c)(2)]
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☒ A translation of the International Application into English [35 U.S.C. 371(c)(2)].

7. ☒ Amendments to the claims of the International Application under PCT Article 19 [35 U.S.C. 371(c)(3)]
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 [35 U.S.C. 371(c)(3)].

9. ☐ An oath or declaration of the inventor(s) [35 U.S.C. 371(c)(4)].

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 [35 U.S.C. 371(c)(5)].

Items 11 - 16 below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information: PCT/IPEA/416; PCT/IPEA/409; 5 pages of amended claims Drawings (14 sheets)

U.S. APPLN. NO. (IF KNOWN SEE 37 C.F.R. 1.5) 09/926323	INTERNATIONAL APPLICATION NO. PCT/EPOO/03347	ATTORNEY DOCKET NO. 100564-00082
		DATE: October 15, 2001

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee [37 C.F.R. 1.492(a)(1)-(5)]:		
Search Report has been prepared by the EPO or JPO.....$890.00		
International preliminary examination fee paid to USPTO (37 C.F.R. 1.482).....$690.00		
No international preliminary examination fee paid to USPTO (37 C.F.R. 1.482) but international search fee paid to USPTO [37 C.F.R. 1.445(a)(2)].....$710.00		
Neither international preliminary examination fee (37 C.F.R. 1.482) or international search fee [37 C.F.R. 1.445(a)(2)] paid to USPTO.....$1,000.00		
International preliminary examination fee paid to USPTO (37 C.F.R. 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....$ 100.00		

ENTER APPROPRIATE BASIC FEE AMOUNT =				$ 890.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date [37 C.F.R. 1.492(e)].				$	
Claims	Number Filed	Number Extra	Rate		
Total Claims	24 - 20 =	4	X $ 9.00	$ 36.00	
Independent Claims	5 - 3 =	2	X $ 42.00	$ 84.00	
Multiple dependent claim(s) (if applicable)			+ $270.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 1,010.00	
Reduction by one-half for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 C.F.R. 1.9, 1.27, 1.28).				$ 505.00	
SUBTOTAL =				505.00	
Processing fee of $130.00 for furnishing the English translation later the ☐ 20 ☐ 30 months from the earliest claimed priority date [37 C.F.R. 1.492(f)]. +				$	
TOTAL NATIONAL FEE =				$ 505.00	
Fee for recording the enclosed assignment [37 C.F.R. 1.21(h)]. The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. 3.28, 3.31). $40.00 per property +				$	
TOTAL FEES ENCLOSED =				$ 505.00	
				Amount to be refunded	$
				Charged	$

a. ☒ A check in the amount of $505.00 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. 01-2300 in the amount of $ to cover the above fee.
A duplicate copy of this sheet is enclosed.
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 01-2300.

NOTE: Where an appropriate time limit under 37 C.F.R. 1.494 or 1.495 has not been met, a petition to revive [37 C.F.R. 1.137(a) or (b)] must be filed and granted to restore the application to pending status.

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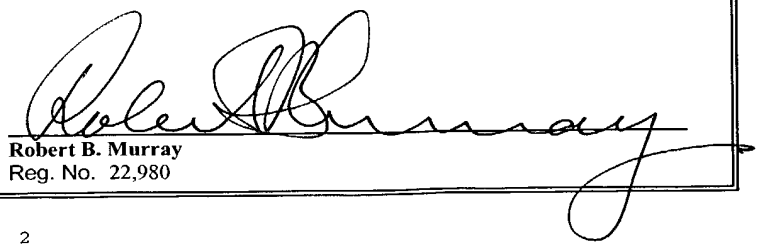
RBM/baw



Robert B. Murray
Reg. No. 22,980

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:
SCHMITT et al.

Appln. No.: PCT/EP00/03347

Filed: Concurrently herewith Attorney Dkt. No.: 100564-00082

For: DIAGNOSTIC AND THERAPEUTIC USE OF ANTIBODIES AGAINST THE UROKINASE RECEPTOR

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231 October 15, 2001

Sir:

Prior to calculation of the filing fees and initial examination of the application, please amend the above-identified application as follows:

IN THE SPECIFICATION:

Before Line 1, page 1 insert

--CROSS-REFERENCE TO RELATED APPLICATION

This application is a National Stage entry of International Application No. PCT/EP00/03347, filed April 13, 2000, the entire specification claims and drawings of which are incorporated herewith by reference.

IN THE CLAIMS:

Please amend claims 3-9; 12; 16-18; 22 and 23 as follows:

3. (Amended) Method as claimed in claim 1, characterized that antibodies or antibody fragments or/and receptor ligands are used as cell-specific binding molecules.

4. (Amended) Method as claimed in claim 1, characterized in that a second cytokeratin-specific binding molecule is used.

5. (Amended) Method as claimed in claim 1, characterized in that the binding molecules are indirectly labeled.

6. (Amended) Method as claimed in claim 1, characterized in that the binding molecules are directly labeled.

7. (Amended) Method as claimed in claim 1, characterized in that the sample is evaluated quantitatively by a confocal laser scanning microscope or by a fluorescence microscope.

8. (Amended) Method as claimed in claim 1 characterized in that the sample is evaluated by parallel or/and sequential determination of the fluorescence of the various labeling groups.

9. (Amended) Method as claimed in claim 1, additionally comprising a characterization of cells identified by reaction with the binding molecules.

12. (Amended) Use of the method as claimed in claim 1 to detect micrometastases in biological samples.

16. (Amended) Use as claimed in claim 14 in an ELISA.

17. (Amended) Use as claimed in claim 14 in a double-fluorescence detection method.

18. (Amended) Use as claimed in claim 13, characterized in that the antibody is selected from the monoclonal antibody IIIF10, fragments thereof or antibodies and antibody fragments having an equivalent binding specificity.

22. (Amended) Recombinant polypeptide as claimed in claim 20, characterized in that it is a humanized antibody fragment.

23. (Amended) Recombinant polypeptide as claimed in claim 20, characterized in that it is coupled to an effector group.

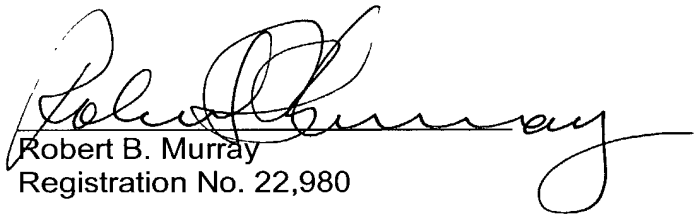
Please add the following claim:

--24. Use of the reagent kit as claimed in claim 11 to detect micrometastases in biological samples.--

REMARKS

Claims 1-24 are pending in this application. By this Amendment, claims 3-9; 12; 16-18; 22 and 23 are amended to correct the multiple dependency thereof and to place this application into better condition for examination. No new matter is added.

Respectfully submitted,



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RBM/baw

Claims

1. Method for the quantitative detection of epithelial tumour cells in a biological sample in which the biological sample contains the cells to be detected in a range of $1:10^4$ to $1:10^7$ of the total cells present in the sample comprising the steps:
   (a) preparing a sample to be tested,
   (b) contacting the sample with at least two different binding molecules which recognize the cells to be detected, the binding molecules being each labelled with different fluorescent dyes and one of the binding molecules being specific for the urokinase receptor, and
   (c) determining the fluorescent labels in the sample fixed on a solid phase.

2. Method as claimed in claim 1,
   characterized in that
   the detection is carried out in a bone marrow sample.

3. Method as claimed in [one of the claims 1 to 2], claim 1
   characterized in that
   antibodies or antibody fragments or/and receptor ligands are used as cell-specific binding molecules.

4. Method as claimed in [one of the claims 2 to 3], claim 1
   characterized in that
   a second cytokeratin-specific binding molecule is used.

5. Method as claimed in [one of the claims 1 to 4], claim 1
characterized in that
the binding molecules are indirectly labelled.

6. Method as claimed in [one of the claims 1 to 4], claim 1
characterized in that
the binding molecules are directly labelled.

7. Method as claimed in [one of the claims 1 to 6], claim 1
characterized in that
the sample is evaluated quantitatively by a confocal laser scanning microscope or by a fluorescence microscope.

8. Method as claimed in [one of the claims 1 to 7], claim 1
characterized in that
the sample is evaluated by parallel or/and sequential determination of the fluorescence of the various labelling groups.

9. Method as claimed in [one of the claims 1 to 8], claim 1
additionally comprising a characterization of cells identified by reaction with the binding molecules.

10. Method as claimed in claim 9,
characterized in that
the characterization comprises a site-specific determination of the fluorescent label.

amended page

11. Reagent kit for the quantitative detection of cells in a biological sample comprising
    (a) a first binding molecule which recognizes the cells to be detected and a first fluorescent labelling group, the binding molecule being specific for the urokinase receptor,
    (b) a second binding molecule which recognizes the cells to be detected and a second fluorescent labelling group, the first and the second binding molecule and the first and the second fluorescent labelling group being different and
    (c) means for fixing cells on a solid phase.

12. Use of the method as claimed in [one of the claims 1 to 10 or the reagent kit as claimed in claim 11] claim 1 to detect micrometastases in biological samples.

13. Use of an antibody which is directed against the epitope 52-60 of the urokinase receptor (uPAR) or of an antigen binding fragment thereof to produce a diagnostic agent directed against uPAR on tumour cells to make a prognosis of the course of malignant diseases.

14. Use as claimed in claim 13 as a diagnostic agent to detect tumour cells in a biological sample.

15. Use as claimed in claim 14 to detect disseminated tumour cells in bone marrow.

amended page

16. Use as claimed in [one of the claims 14 to 15] (claim 14) in an ELISA.

17. Use as claimed in [one of the claims 14 to 15] (claim 14) in a double-fluorescence detection method.

18. Use as claimed in [one of the claims 13 to 17] (claim 13), **characterized in that**
the antibody is selected from the monoclonal antibody IIIF10, fragments thereof or antibodies and antibody fragments having an equivalent binding specificity.

19. Recombinant nucleic acid which codes for a polypeptide having antibody properties comprising
(a) a CDR3-VH sequence coding for the amino acid sequence (I):
D G S M G G F D Y
or/and
(b) a CDR3-VL sequence coding for the amino acid sequence (II):
L Q H W N Y P Y T.

20. Recombinant polypeptide having antibody properties comprising:
(a) a CDR3-VH amino acid sequence (I):
D G S M G G F D Y
or/and
(b) a CDR3-VL amino acid sequence (II):
L Q H W N Y P Y T.

21. Recombinant polypeptide as claimed in claim 20,
characterized in that
it is an scFv antibody fragment.

22. Recombinant polypeptide as claimed in claim 20 [or 21],
characterized in that
it is a humanized antibody fragment.

23. Recombinant polypeptide as claimed in [one of the claim 20 claims 20 to 22],
characterized in that
it is coupled to an effector group.

14/pRT

Diagnostic and therapeutic use of antibodies against the urokinase receptor

Description

The invention concerns a method and a reagent kit for detecting cells in a biological sample using a double-fluorescence technique.

The reliable detection of disseminated tumour cells which have escaped from a solid tissue structure (micrometastases) is of major importance for tumour diagnostics and treatment. Hence various methods have been developed over the past years to detect such individual disseminated tumour cells in body fluids or tissue samples. They can for example be detected by selectively labelling the rare cells by means of immunocytochemical methods in which case enzymatic labelling groups such as alkaline phosphatase are often used. Double labelling techniques are also known.

A publication by Schlimok et al. (Proc. Natl. Acad. Sci. USA 84 (1987) 8672-8676) describes the detection of micrometastatic tumour cells in bone marrow by means of a double-labelling technique in which a cytokeratin 18 antibody which is specific for cells of epidermal origin and a leucocyte antibody are used. In this method alkaline phosphatase and a radioactive labelling group (^{125}I) are used. Since there are drawbacks associated with the use of radioactive labelling groups, this method is not suitable for clinical practice.

Funke et al. (Int. J. Cancer 65 (1996), 755-761)

describe the detection of micrometastases in bone marrow by means of a double-labelling technique using a cytokeratin 18 antibody and an E-cadherin antibody. Both antibodies are detected by means of alkaline phosphatase as an enzymatic labelling group and two differently coloured chromogenic substrates. However, the sequential detection of both antibodies by different chromogenic substrates is complicated and hence less suitable for clinical practice.

Heiss and co-workers (Heiss et al., Nature Med. 1 (1995), 1035-1039 and Allgayer et al., J. Histochem. Cytochem. 45 (1997), 203-212) detect disseminated tumour cells in bone marrow by means of a double-labelling method based on the simultaneous detection of cytokeratin 18 and the uPA receptor (uPAR). For this cells bound and fixed on a microscope slide are incubated with a biotinylated cytokeratin-specific antibody and subsequently with a conjugate of alkaline phosphatase and streptavidin. An enzymatic staining reaction is carried out using the immobilized alkaline phosphatase and a chromogenic substrate to form a dark-red stain. In addition a monoclonal antibody against uPAR is used which is labelled with a gold-conjugated secondary antibody and subsequently subjected to a silver enhancement reaction which results in a black stain. The microscope slides are then manually and visually screened for the stains (dark-red/black) under a microscope but a double stain is extremely difficult to detect.

The object of the present invention was to carry out a method for the detection of cells in particular of rarely occurring cells such as tumour cells in a biological sample e.g. bone marrow which at least

partially eliminates the disadvantages of the prior art. In particular the method should at the same time be highly sensitive and enable a trouble-free evaluation.

This object is achieved by a method for detecting cells in a biological sample which comprises the following steps:

(a) preparing a sample to be tested,
(b) contacting the sample with at least two different binding molecules which recognize the cells to be detected, the binding molecules being each labelled with different fluorescent dyes and
(c) determining the fluorescent labels in the sample fixed on a solid phase.

The method according to the invention is suitable for detecting rarely occurring cells in a fixed biological sample. In this connection "rarely occurring" in the sense of the present invention means that the expected frequency of the cells to be detected is in the range of $1:10^4$ to $1:10^7$ of the total number of cells present in the sample to be detected. Examples of such rarely occurring cells are tumour cells in a blood or bone marrow sample. Other types of rarely occurring cells can of course also be detected if the cell-specific determinants and specific binding molecules are selected accordingly.

The double-fluorescent staining technique of the method according to the invention allows a rapid and accurate identification of the cells to be detected. In addition the use of different fluorescent labels that can be preferably detected concurrently enables antigens to be analysed that are co-located in as well as on the cell

(such as e.g. cytokeratin 8/18, p53, PAI-2 and in particular the urokinase receptor uPAR). This has previously been very difficult with the known methods especially in the case of tissue samples such as bone marrow aspirates. A further major advantage of the newly developed method is that it allows a quantitative determination of the number and intensity of fluorescent cells for example with a confocal laser scanning microscope.

Step (a) of the method according to the invention comprises the provision of a biological sample to be tested. For this a sample is taken from the patient e.g. from a body fluid such as blood or from a tissue such as bone marrow. The method according to the invention is particularly preferably used to detect disseminated tumour cells of epidermal origin in the bone marrow. The bone marrow can be taken from the iliac crest bone. Mononuclear cells including tumour cells are then preferably concentrated in the sample. This concentration can be carried out by known methods for example by density gradient centrifugation e.g. Ficoll in which a separation of erythrocytes and granulocytes occurs.

The sample to be tested preferably contains at least 10^6 cells in order to enable a reliable detection of rare cells. The sample particularly preferably contains 10^6 to 10^9 and in particular $5x10^6$ to $5x10^7$ cells.

According to step (b) the sample is contacted with at least two different binding molecules that are directed against the cells to be detected. The binding molecules are preferably antibodies or antibody fragments and in

particular monoclonal antibodies or antibody fragments. However, it is also possible to use ligands of receptors such as the uPA receptor that specifically occur in the cells to be detected. Examples of such ligands are linear or/and cyclic peptides or peptide mimetics which can carry a fluorescent label.

The sample is preferably contacted with the fluorescent-labelled binding molecules after the cells have been fixed on a solid phase. This fixation can be carried out by known methods e.g. using formaldehyde or glutardialdehyde. A microscope slide can for example be used as the solid phase.

If necessary the cells present in the sample to be tested can be permeabilized using a detergent such as a saponin. This enables the binding molecules to also bind to intracellular determinants.

For the detection of tumour cells the binding molecules are directed against determinants which only occur in tumour cells or are present at an increased concentration in tumour cells in the sample to be tested but do not occur in normal cells or only in a low concentration. A structure from the interior of the cells e.g. a cytokeratin is preferably selected as the first determinant. Cytokeratins are specific components of the cytoskeleton of epithelial cells and are not expressed in mononuclear blood or bone marrow cells which are of mesenchymal origin. Hence the presence of cytokeratins in cells which have been taken from blood and bone marrow indicates the presence of epithelial tumour cells. Examples of suitable anti-cytokeratin antibodies are the antibody A45B/B3 (Micromet GmbH,

Munich, Germany) or the antibody CK2 (Boehringer Mannheim GmbH, Mannheim, Germany). Other detection antibodies directed against intracellular tumour-associated antigens are known and are commercially available from various companies.

A structure on the cell surface such as a membrane receptor is preferably selected as the second determinant. The urokinase receptor (uPAR) is a particularly preferred tumour-specific determinant. This receptor can for example be detected using anti-uPAR antibodies such as IID7 and IIIF10 (Luther et al., Am. J. Path. 150 (1997), 1231-1244). Those anti-uPAR antibodies are preferably selected which have an affinity for a tumour cell-specific uPAR which is at least comparable to that for a uPAR from normal cells. Examples of anti-uPAR antibodies which also bind to tumour cells with high affinity are antibodies which recognize the epitope 52-60 of uPAR such as the above-mentioned antibody IIIF10.

In contrast other anti-uPAR antibodies often only poorly recognize uPAR on tumour cells.

On the other hand uPAR can also be detected with fluorescent-labelled receptor ligands e.g. urokinase, urokinase fragments or urokinase peptides. Such detection methods are described for example by Chucholowski et al. (Fibrinolysis 6, Suppl. 4 (1992), 95-102), Ciccocioppo et al. (J. Histochem. Cytochem. 45 (1997), 1307-1313) and Luther et al. (Am. J. Pat. 150 (1997), 1231-1242).

At least two different fluorescent labelling groups are used in the method according to the invention. It is advantageous to use fluorescent labelling groups which have emission spectra (e.g. red/green) that can be distinguished from one another. Examples of suitable fluorescent dyes are fluorescein and derivatives thereof, phycoerythrin, rhodamine, TRITC-amines, Texas Red® amines, CY3 and CY5 as well as Alexa® 488 and Alexa® 568 (Molecular Probes). The fluorescent dyes can be directly e.g. covalently conjugated with the primary binding molecules that are specific for the cells to be detected. This is referred to as a direct label. On the other hand the fluorescent dyes can be conjugated to secondary binding molecules which are in turn directed against the primary binding molecules. This is referred to as an indirect label. Both labelling methods or combinations thereof can be used in the method according to the invention.

The various binding molecules can be sequentially or concurrently incubated with the cell. An incubation with several binding molecules in parallel (primary binding molecules and optionally secondary binding molecules in the case of an indirect label) leads to a considerable time saving.

The sample is evaluated by determining the fluorescence after exciting the fluorescent labelling groups. A confocal laser scanning microscope or a fluorescence microscope is particularly preferably used for this which enable an evaluation of the sample by concurrent or/and sequential determination of the various fluorescent labelling groups.

The double-fluorescence labelling technique according to the invention additionally enables a characterization of the cells identified as positive by reaction with the binding molecules. This characterization can comprise site-specific or/and quantitative evaluation of the label. Hence individual cells can be "scanned" by determining the label in several e.g. 10 to 50 planes of sections through the cell at distances of for example 0.1 to 1 μm. In addition the determinants in the cell that have reacted with the binding molecules can be determined quantitatively on the basis of a standard curve which has been constructed by measuring microparticles of a defined size and a defined amount of fluorescent dye.

The method according to the invention allows valuable diagnostic data to be obtained from tumour patients and hence enables a sensitive prognosis to be made for patients after operation of a primary tumour.

Finally the invention concerns a reagent kit for the detection of cells in a biological sample comprising

(a) a first binding molecule which recognizes the cells to be detected and a first fluorescent labelling group,
(b) a second binding molecule which recognizes the cells to be detected and a second fluorescent labelling group, the first and the second binding molecule and the first and the second fluorescent labelling group being different and
(c) means for fixing cells on a solid phase.

It was surprisingly found that uPAR antibodies which are directed against the epitope 52 - 60 of uPAR recognize a uPAR having a glycostructure that occurs in tumour cells

i.e. bind to a uPAR expressed by tumour cells with an at least comparable affinity to a uPAR expressed by normal cells. In contrast other anti-uPAR antibodies e.g. HD13.1 (Todd et al., CD87 workshop panel report. In: Kishimoto T. et al., publ. Leucocyte Typing VI, New York & London, Garland Publishing, Inc. 1997; 1016-1020) only have a low affinity for uPAR from tumour cells.

Hence the invention concerns the use of an antibody or of an antigen-binding fragment thereof (preferably of a monoclonal antibody or of an antigen-binding fragment thereof) which is directed against the epitope 52 to 60 of uPAR to produce a diagnostic or therapeutic agent directed against uPAR on tumour cells. Such antibodies like the known monoclonal antibody IIIF10 (Luther et al. (1997), supra) or antibodies having an equivalent binding specificity such as chimerised or humanized antibodies or corresponding recombinant or proteolytic antibody fragments, e.g. single-chain antibody fragments, recognize a uPAR expressed by tumour cells with an adequate affinity for diagnostic and therapeutic purposes.

Furthermore it was surprisingly found that such antibodies or fragments thereof can be used as a diagnostic agent to predict the course of malignant diseases especially in the case of tumours e.g. breast carcinomas. In tumour samples from over 200 examined female breast carcinoma patients it was found that the binding of the antibody IIIF10 or of a corresponding antibody with an equivalent binding capability has a significant prognostic relevance for the course of the disease i.e. absence of recidivity or death. In this connection high antigen values indicate a shorter absence of recidivity or an earlier death. Such a

prognostic significance was not found with antibodies which are directed against other regions of uPAR.

Due to their high affinity for tumour uPAR these antibodies or fragments thereof are also suitable as diagnostic agents for detecting tumour cells in a biological sample and in particular for detecting disseminated tumour cells in bone marrow. Such detection methods can for example be carried out as an ELISA or as previously elucidated in detail double-fluorescence detection methods.

Moreover antibodies which are directed against the epitope 52 to 60 of uPAR or fragments thereof are suitable for preparing a therapeutic agent with for example selective function blocking activity in tumour cells. In addition the antibodies or fragments thereof can be used in the form of conjugates with a cytotoxic group to inhibit the growth of or kill tumour cells. Examples of suitable cytotoxic groups are radioactive groups, toxins and cell growth inhibitors. For therapeutic applications it is preferable to use chimeric antibodies with humanized constant domains the production of which is described for example in EP-B-0 120 694.

Yet a further subject matter of the invention are recombinant nucleic acids which code for a polypeptide with antibody properties and contain the CDR3-VH sequence or/and the CDR3-VL sequence of the antibody IIIF10. The CDR3 region of the VH cDNA is shown in SEQ ID NO.1/2 from nucleotide 295 to 321 (corresponding to amino acid 99 to 107). The CDR3 region of the VL cDNA is shown in SEQ ID NO.3/4 from nucleotide 265 to 291 (amino acid 89 to 97). In addition the nucleic acids preferably

contain the sections of VH or/and VL cDNA coding for the CDR1 and/or CDR2 regions. The sequences for the CDR1-VH region are shown in SEQ ID NO.1/2 from nucleotide 91 to 105 (corresponding to amino acid 31 to 35, i.e. SYDIN). In SEQ ID NO.3/4 the CDR1 region of the VL cDNA extends from nucleotide 70 to 102 (corresponding to amino acid 24 to 34, i.e. KAS...TVA). The CDR2 region of the VH cDNA extends from nucleotide 148 to 198 (amino acid 50 to 66, i.e. WIF...FKD) in SEQ ID NO.1/2. The CDR2 region of the VL cDNA extends from nucleotide 148 to 168 (corresponding to amino acid 50 to 56, i.e. LASNRHT) in SEQ ID NO.3/4.

Thus the invention concerns in particular recombinant nucleic acids which code for a polypeptide having antibody properties comprising

(a) a CDR3-VH sequence coding for the amino acid sequence (I):

D G S M G G F D Y

or/and

(b) a CDR3-VL sequence coding for the amino acid sequence (II):

L Q H W N Y P Y T

Furthermore the invention concerns recombinant polypeptides having antibody properties comprising

(a) a CDR3-VH amino acid sequence (I):

D G S M G G F D Y

or/and

(b) a CDR3-VL amino acid sequence (II):

L Q H W N Y P Y T

The recombinant nucleic acids and polypeptides preferably contain the CDR3 regions of the VH as well as

of the VL sequence. The recombinant polypeptides are particularly preferably single-chain antibodies e.g. scFv antibody fragments. In the recombinant polypeptides the framework domains which are not directly responsible for antigen binding are preferably replaced by corresponding human sequences such that humanized antibody fragments are formed. The recombinant polypeptides according to the invention can be coupled with effector groups i.e. cytotoxic groups for therapeutic applications or/and detection groups for a tumour imaging.

The invention is further elucidated by the following figures and examples.

Figure 1: shows a diagrammatic view of the scanning of a cell in a laser microscope.

a) A total of 30 serial sections with a spacing of 0.5 µm is prepared from a ca. 15 µm large tumour cell.

b) The fluorescence is measured in each plane of the section and then all fluorescence values are added.

c) The total fluorescence is calculated from a standard curve (latex microparticles containing a defined amount of fluorochrome).

Figure 2: shows the result of the fluorescence staining of a tumour cell with the anti-cytokeratin antibody A45 B/B3 and Alexa 488 as a fluorescent dye.

a) The sequence of images shows 24 photographs of a scan procedure in which a ca. 12 µm breast carcinoma cell (ZR75) was measured in

section planes with a spacing of 0.5 µm in each case.

b) shows an extended focus photograph in which the total intensity of the entire scan (a) has been projected onto a single image plane.

Figure 3: shows the result of an indirect fluorescence staining with A45B/B3 as the primary antibody and a secondary antibody conjugated with Alexa 488 (enlargement x63),

a) transmission image

b) a cytokeratin-positive cell in the bone marrow smear of a female patient with breast carcinoma.

Figure 4: shows the result of a direct fluorescence staining with a conjugate of the antibody A45B/B3 and the fluorescent dye Alexa 488 (enlargement x63),

a) transmission image

b) cytokeratin detection in a mixed preparation of MCF7 tumour cells and peripheral blood lymphocytes (1:20).

Figure 5: shows the result of a direct fluorescence staining with a conjugate of the anti-uPAR antibody IIIF10 and the fluorescent dye Alexa 568 (enlargement x63),

a) transmission image

b) uPAR receptor detection in a mixed preparation of MCF7 tumour cells and peripheral blood lymphocytes (1:20).

Figure 6: shows the result of a direct double-fluorescence staining with the conjugates A45B/B3-Alexa 488 (anti-cytokeratin) and IIIF10-Alexa 568 (anti-uPAR),

a) transmission image
b) cytokeratin detection
c) uPAR receptor detection

Figure 7: shows the result of the measurement of a tumour cell in the bone marrow (enlargement x63),

a) transmission image (Nomarski optics)
b) reaction of the cell with a conjugate of Alexa 488 and an anti-cytokeratin antibody.
c) reaction of the cell with a conjugate of Alexa 568 and a uPAR antibody. The cell nucleus is not stained. The reaction of the anti-uPAR antibody is mainly limited to the cell membrane. The uPAR-positive bone marrow cell which is negative for cytokeratin is shown on the bottom right. All other cells are uPAR-negative.

Figure 8: shows the influence of uPA on the uPAR determination

a) the UPA/uPAR ratio in tumour extracts from 599 breast carcinoma patients,
b) the determination of uPAR in the presence of different amounts of uPA.

Figure 9: shows the uPAR-antigen content in various cells determined by different test procedures:
IIIF10/HU277 black, HD13.1/HU277: dark grey,

ADI light grey

a) normal cells
b) well-differentiated tumour cells
c) poorly-differentiated tumour cells

Figure 10: shows the prognostic relevance of the uPAR antigen content determined by various test procedures in 203 breast carcinoma patients

a) IIIF10/HU277
b) HD13.1/HU277
c) ADI

Figure 11: shows the dose-dependent inhibition of tumour growth of human breast cancer in naked mice by administering the antibody IIIF10.

Figure 12: shows the binding of scFv IIIF10 to immobilized antigens.

Figure 13: shows the inhibition of the binding of IIIF10 (monoclonal antibody/moab and scFv) to uPAR by peptides.

SEQ ID NO 1/2: shows the nucleotide sequence of the cDNA coding for the VH chain of IIIF10 VH and the corresponding amino acid sequence.

SEQ ID NO 3/4: shows the nucleotide sequence of the cDNA coding for the VL chain of IIIF10 and the corresponding amino acid sequence.

Examples

1. Double-fluorescence determination of tumour cells

1.1 Material

The monoclonal mouse antibody A45B/B3 (Kaspar et al., Eur. J. Cancer Clin. Oncol 23, (1987), 137-147) is directed against the cytokeratin filaments 8, 18 and 19 (CK 8, 18, 19). This antibody was directly conjugated with the fluorochrome ALEXA 488 from Molecular Probes. The uPA receptor is specifically detected by the monoclonal mouse antibody IIIF10 (Luther et al. (1997), supra) (epitope 52 to 60). The monoclonal antibodies HD13.1 and IID7 (Luther et al. (1997), supra) (epitope 125 to 132) as well as the polyclonal rabbit antibody #399R (Stahl et al., Cancer Res. 54 (1994), 3066-3071) and the chicken antibody HU277 (Magdolen et al., Electrophoresis 16 (1995), 813-816) are available as additional uPA receptor antibodies. All monoclonal antibodies against the uPA receptor were directly conjugated with the fluorescent dye ALEXA® 568.

Table 1 Directly conjugated antibodies that were used

Monoclonal antibody	Antigen	directly conjugated with	excitation range in the CLSM*	Manufacturer
mAb II D 7 (mouse)	uPAR, domain 2	ALEXA 568 (™Molecular Probes)	568 nm	Pathology Dresden and Gynaecological Hospital Munich
mAb III F 10 (mouse)	uPAR, domain 1	ALEXA 568 (™Molecular Probes)	568 nm	Pathology Dresden and Gynaecological Hospital Munich
mAb HD 13.1 (mouse)	uPAR, domains 2+3	ALEXA 568 (™Molecular Probes)	568 nm	Immunology Heidelberg
mAB A45 B/B3 (mouse)	cytokeratin 8/9/18	ALEXA 488 (™Molecular Probes)	488 nm	Micromet Munich

(*CLSM = confocal laser scanning microscope)

1.2 Bone marrow preparations

A Jamshidi puncture is carried out in the operating theatre. 4-6 ml bone marrow is taken from both iliac crests. The tumour cells in the mononuclear cell fraction are concentrated by means of a Ficoll gradient. 8 to 12 cytospins (10^6 cells by cytospin) are prepared per patient. After air-drying the preparations are fixed and permeabilized.

1.3 Fixation and permeabilization

1. Fixation in 4 % paraformaldehyde (PFA) for 30 min.
2. Wash three times in phosphate-buffered saline/1 % bovine serum albumin (PBS/BSA).
3. Permeabilize in 0.025 % saponin for 45 min.

4. Wash three times in PBS/1 % BSA.

1.4 Double-labelling of the cytokeratin and uPA receptor

1.4.1. Indirect method

1. Incubate overnight with the primary mouse antibody A45B/B3 (final concentration 0.004 mg/ml) in PBS/ 1 % BSA.
2. Wash three times with PBS/1 % BSA.
3. Incubate for two hours with the second primary rabbit antibody #399 R (final concentration 0.05 mg/ml) diluted in PBS/1 % BSA.
4. Wash three times in PBS/1 % BSA.
5. Secondary antibody goat anti-mouse-Alexa 488 (final concentration 0.02 mg/ml) diluted in PBS/1 % BSA, incubation period 30 min.
6. Wash three times in PBS/1 % BSA.
7. Secondary antibody goat anti-rabbit-Alexa 568 (final concentration 0.02 mg/ml) diluted in PBS/1 % BSA, incubation period 30 min.
8. Wash three times in PBS/1 % BSA.
9. Cover with 5 μl PBS/1 % BSA and examine under a microscope.

1.4.2 Direct method

1. Incubate for 1 hour with the antibody A45B/B3-Alexa 488 (final concentration 0.0014 mg/ml) diluted in PBS/1 % BSA.
2. Wash three times in PBS/1 % BSA.
3. Incubate for 1 hour with the antibody IIIF10-Alexa 568 (final concentration 0.003 mg/ml) diluted in PBS/1 % BSA.

4. Wash three times in PBS/1 % BSA.
5. Cover with 5 µl PBS/ 1 % BSA and examine under a microscope.

1.5 Quantification

The antigens reacting with the fluorescent antibody are visualized in a confocal laser scanning microscope at an excitation range of 488 nm and 568 nm. The tumour cells are divided into 20 to 30 planes of section by scanning the cell in a laser microscope i.e. by layering in 0.5 µm steps. All fluorescences are detected and the sum of these measurements is calculated. The antigens in the tumour cell which have reacted with the antibody can be quantified on the basis of a standard curve which has been previously constructed by measuring latex beads containing a defined amount of fluorescent dye.

Figure 1 shows a diagram of the principle of the scanning procedure used to localize and quantify the fluorescent label. Figures 2 to 7 show examples of results for the practical application of the method according to the invention.

2. Tumour specificity of the monoclonal antibody IIIF10

Two different ELISA systems were developed for the detection of uPAR antigen:

1) Capture antibody: polyclonal chicken antibody HU277 (Magdolen et al. (1995), supra); detection antibody: monoclonal antibody IIIF10 (Luther et al. (1997), supra)

2) Capture antibody: polyclonal chicken antibody HU277; monoclonal antibody HD13.1 (Todd et al. (1997), supra).

These ELISA systems were compared with a commercially available ELISA (ADI) for uPAR (American Diagnostica Inc. Greenwich, CT, USA).

The tested ELISA systems were matched using recombinant affinity-purified human uPAR (rec-uPAR) expressed in CHO cells. All three ELISA systems exhibited a comparable linearity and sensitivity towards rec-uPAR.

In further experiments it was demonstrated that the actual uPAR antigen content on cells can also be determined in the presence of an up to six-fold excess of uPAR. The recovery was > 95 % in the case of IIIF10/HU277 and the HD13.1/HU277 test and > 80 % in the case of the ADI test. The uPA/uPAR ratio in 599 analysed tumour extracts is typically < 3 in 95 % of the cases (tests with ADI-UPA and ADI-uPAR-ELISA). These results are shown in figure 8.

Subsequently the uPAR antigen contents were determined in lysates of various cell types. This showed that the determination of uPAR antigen in non-malignant cells (e.g. keratinocytes [HaCaT], endothelial cells from the umbilical cord [HUVEC], epithelial cells from the breast [HMEC] gave comparable results in all three ELISA systems. In contrast the situation was quite different in the case of tumour cell lines. In well-differentiated breast carcinoma cells only the IIIF10/HU277 ELISA detected significant amounts of tumour-associated uPAR whereas in poorly-differentiated breast carcinoma cell lines the IIIF10/HU277 and the ADI-ELISA gave comparable

values. The HD13.1/HU277-ELISA detected too little uPAR in well-differentiated as well as in poorly-differentiated carcinoma cells. The data are shown in figure 9.

3. Prognostic relevance of the monoclonal antibody IIIF10

In a clinical study the uPAR antigen content was determined using all three ELISA systems described in example 2 in tumour samples from over 200 breast carcinoma patients. This showed that the antigen values measured with the IIIF10/HU277-ELISA have a significant prognostic relevance for the course of the disease i.e. for the absence of recidivity or death. Such a prognostic relevance was not found with the two other ELISA systems. The data are shown in figure 10.

4. In vivo effect of the monoclonal antibody IIIF10

4 to 6 week old Balb/C/3 naked mice were injected on the right flank with $6x10^6$ human breast cancer cells MDA-MB231 (Price et al., Cancer Res. 50 (1990), 717-721) in a total volume of 300 µl. Before injection the cancer cells were mixed in each case with 200 µg of the murine monoclonal antibody IIIF10 in PBS, pH 7.4. Subsequently the mice were treated intraperitoneally with the monoclonal antibody IIIF10 at a dose of 2 mg/kg body weight or 10 mg/kg body weight in an injection volume of 300 µl. The volume of the primary tumours in cm^3 occurring in the mice was determined after four weeks by measuring the two largest diameters of the tumours. PBS pH 7.4 was administered to the control mice, each group consisted of six mice.

The results are shown in fig. 11. It can be seen that the administration of the antibody greatly reduced the growth of primary tumours. The inhibition of growth was even more pronounced when 10 mg/kg body weight was administered than when a dose of 2 mg/kg body weight was administered.

5. Preparation of recombinant monoclonal antibody IIIF10

mRNA from hybridoma cells producing IIIF10 was isolated and transcribed into cDNA. The cDNA fragments coding for the variable regions of the heavy (VH) and the light (VL) chain were amplified by RT-PCR using gene-specific primers. The VH and VL gene segments were cloned into a phagemid vector to enable expression of the variable regions as a single-chain antibody (scFv). The scFv molecules were presented by phage display on the surface of filamentous phages as a fusion protein containing the small phage coat protein pIII. Phages which exhibited a functional expression of scFv-FIII10 were selected by specific binding of uPAR. The selected phages were used to infect E. coli cells which enabled the production and secretion of soluble scFv molecules into the culture medium. Figure 12 shows the binding of the scFv supernatant to uPAR immobilized on a solid phase. The binding capability of the antibodies scFv-anti-X and scFv-anti-Y was also tested for control purposes.

In order to further test the binding specificity, peptides were used which had been used to map the epitope of the antibody IIIF10 (Luther et al., J. Pathol 150 (1997), 1231-1244). As can be seen in figure 13 only one peptide the sequence of which contains the complete IIIF10 epitope on uPAR (51-65), can prevent the binding

of the monoclonal antibody and of scFvIIIF10 to uPAR. Another peptide with an incomplete sequence epitope (48 to 59) is > 100-fold less effective. None of the peptides can prevent the binding of a control antibody scFv-anti-X to its target protein X.

The nucleotide sequence of VH cDNA and the corresponding amino acid sequence are shown in SEQ ID NO. 1/2. The nucleotide sequence of the VL cDNA and the corresponding amino acid sequence are shown in SEQ ID No. 3/4.

C l a i m s

1. Method for detecting cells in a biological sample which comprises the following steps:
   (a) preparing a sample to be tested,
   (b) contacting the sample with at least two different binding molecules which recognize the cells to be detected, the binding molecules being each labelled with different fluorescent dyes and
   (c) determining the fluorescent labels in the sample fixed on a solid phase.

2. Method as claimed in claim 1,
characterized in that
tumour cells are detected.

3. Method as claimed in claim 1 or 2,
characterized in that
the detection is carried out in a bone marrow sample.

4. Method as claimed in one of the claims 1 to 3,
characterized in that
antibodies or antibody fragments or/and receptor ligands are used as cell-specific binding molecules.

5. Method as claimed in one of the claims 2 to 4,
characterized in that
a first cytokeratin-specific binding molecule and a second urokinase receptor-specific binding molecule are used.

6. Method as claimed in one of the claims 1 to 5, **characterized in that** the binding molecules are indirectly labelled.

7. Method as claimed in one of the claims 1 to 5, **characterized in that** the binding molecules are directly labelled.

8. Method as claimed in one of the claims 1 to 7, **characterized in that** the sample is evaluated by a confocal laser scanning microscope or by a fluorescence microscope.

9. Method as claimed in one of the claims 1 to 8, **characterized in that** the sample is evaluated by parallel or/and sequential determination of the fluorescence of the various labelling groups.

10. Method as claimed in one of the claims 1 to 9, additionally comprising a characterization of cells identified by reaction with the binding molecules.

11. Method as claimed in claim 10, **characterized in that** the characterization comprises a site-specific or/and quantitative determination of the fluorescent label.

12. Reagent kit for the detection of cells in a biological sample comprising
(a) a first binding molecule which recognizes the

cells to be detected and a first fluorescent labelling group,

(b) a second binding molecule which recognizes the cells to be detected and a second fluorescent labelling group, the first and the second binding molecule and the first and the second fluorescent labelling group being different and

(c) means for fixing cells on a solid phase.

13. Use of the method as claimed in one of the claims 1 to 11 or the reagent kit as claimed in claim 12 to detect micrometastases in biological samples.

14. Use of an antibody which is directed against the epitope 52-60 of the urokinase receptor (uPAR) or of an antigen binding fragment thereof to produce a diagnostic or therapeutic agent directed against uPAR on tumour cells.

15. Use as claimed in claim 14 as a diagnostic agent to predict the course of malignant diseases.

16. Use as claimed in claim 14 as a diagnostic agent to detect tumour cells in a biological sample.

17. Use as claimed in claim 16 to detect disseminated tumour cells in bone marrow.

18. Use as claimed in one of the claims 15 to 17 in an ELISA.

19. Use as claimed in one of the claims 15 to 17 in a double-fluorescence detection method.

20. Use as claimed in claim 14 as a therapeutic agent for blocking the function of tumour cells.

21. Use as claimed in claim 14 in the form of a conjugate with a cytotoxic group to inhibit the growth of or kill tumour cells.

22. Use as claimed in claim 21,
characterized in that
the cytotoxic group is selected from radioactive groups, toxins and inhibitors.

23. Use as claimed in one of the claims 14 to 22,
characterized in that
the antibody is selected from the monoclonal antibody IIIF10, fragments thereof or antibodies or antibody fragments having an equivalent binding specificity.

24. Recombinant nucleic acid which codes for a polypeptide having antibody properties comprising
(a) a CDR3-VH sequence coding for the amino acid sequence (I):
D G S M G G F D Y
or/and
(b) a CDR3-VL sequence coding for the amino acid sequence (II):
L Q H W N Y P Y T.

25. Recombinant polypeptide having antibody properties comprising:
(a) a CDR3-VH amino acid sequence (I):
D G S M G G F D Y
or/and

(b) a CDR3-VL amino acid sequence (II):
L Q H W N Y P Y T.

26. Recombinant polypeptide as claimed in claim 25, **characterized in that**
it is an scFv antibody fragment.

27. Recombinant polypeptide as claimed in claim 25 or 26,
characterized in that
it is a humanized antibody fragment.

28. Recombinant polypeptide as claimed in one of the claims 25 to 27,
characterized in that
it is coupled to an effector group.

Abstract

The invention concerns a method and a reagent kit for detecting cells in a biological sample using a double-fluorescence technique and the diagnostic and therapeutic application of amino acid sequence-specific antibodies against the urokinase receptor having a high affinity for tumour cell-expressed receptors.

Figure 1

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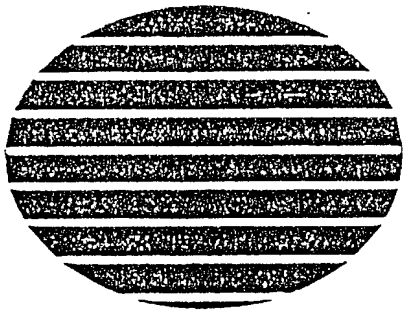

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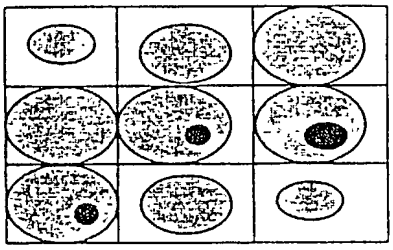

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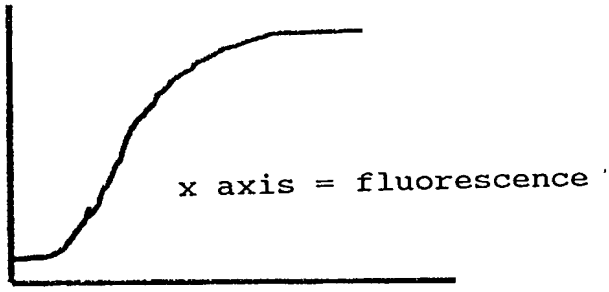

Figure 2

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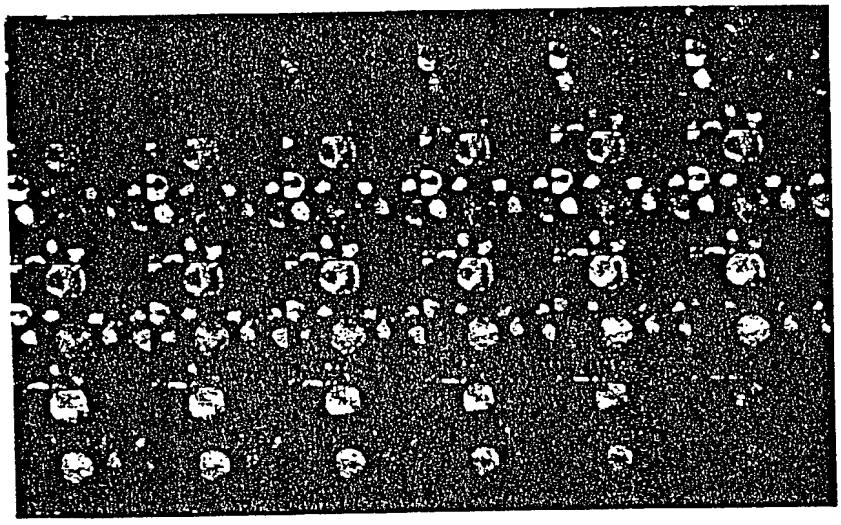

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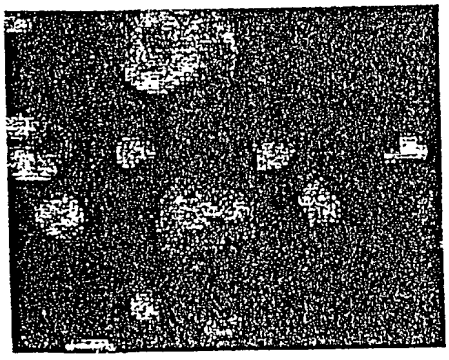

Figure 3

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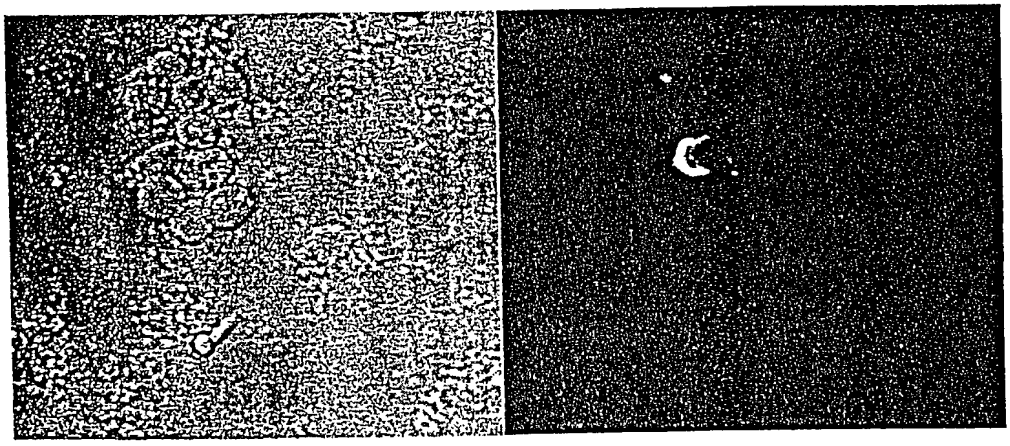

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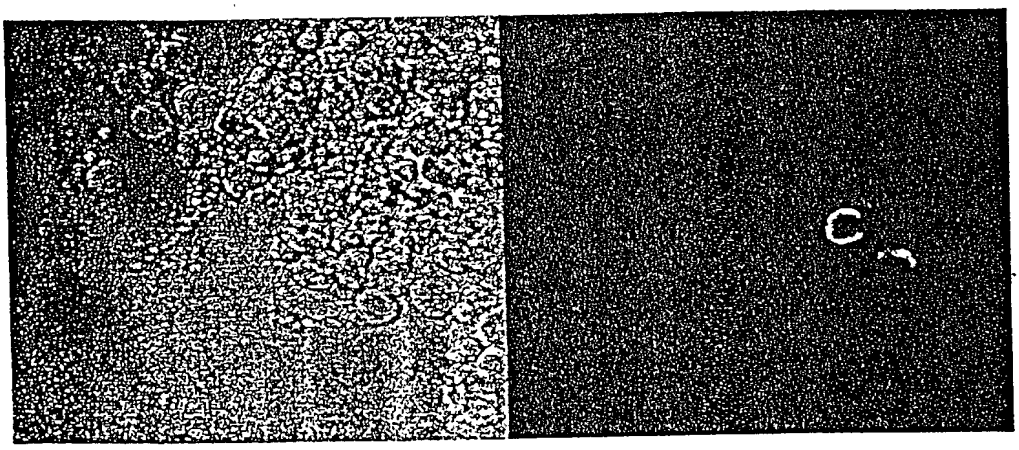
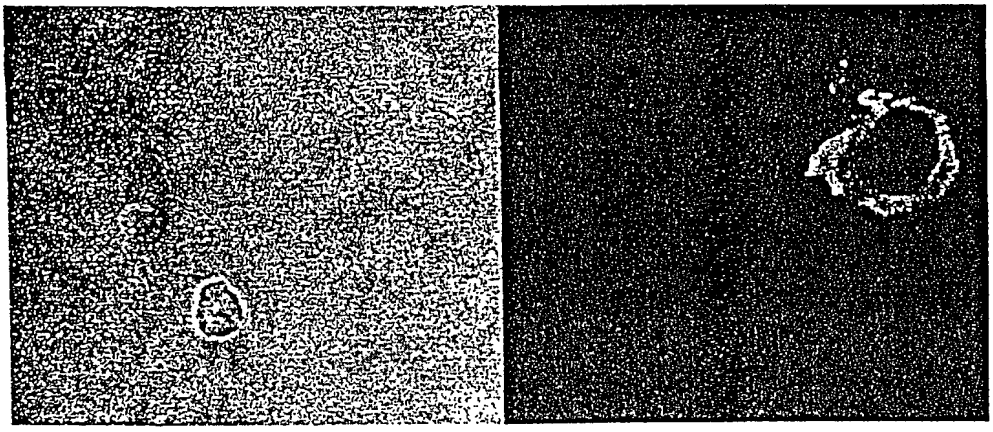


Figure 4

a



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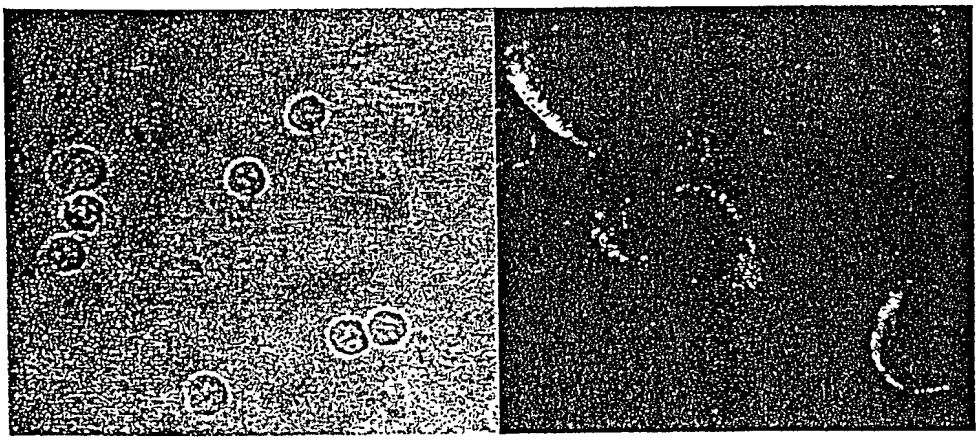

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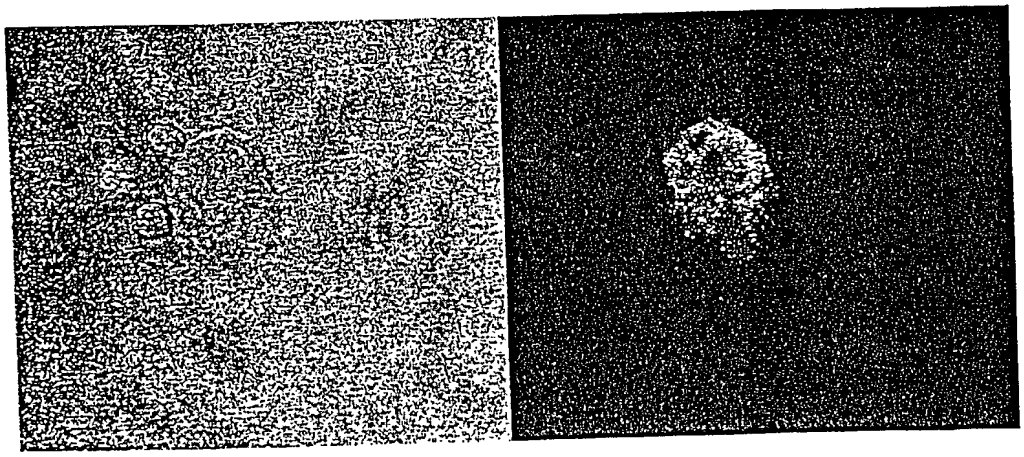

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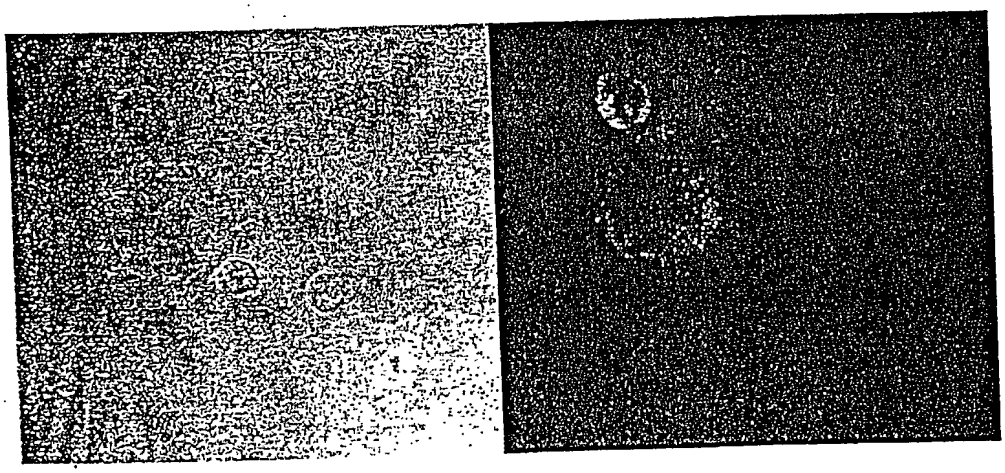

Figure 6

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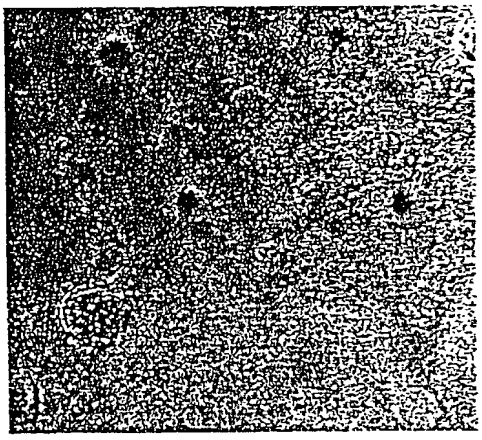

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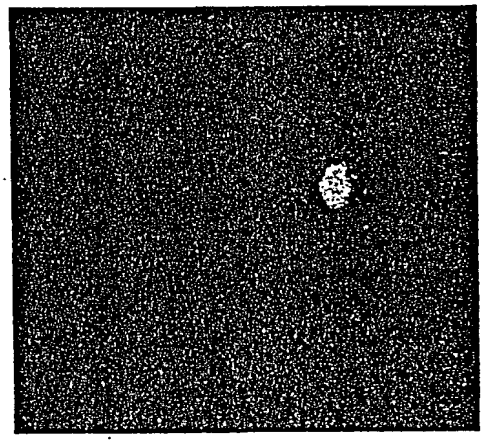

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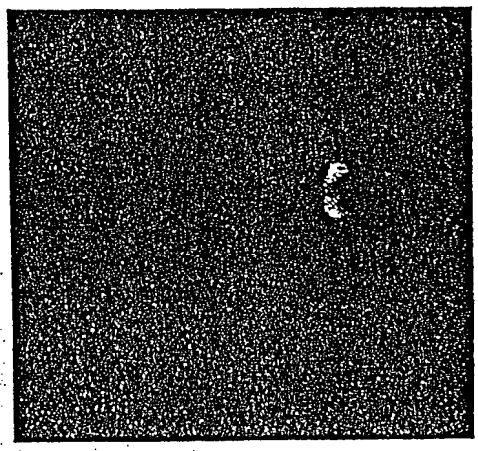
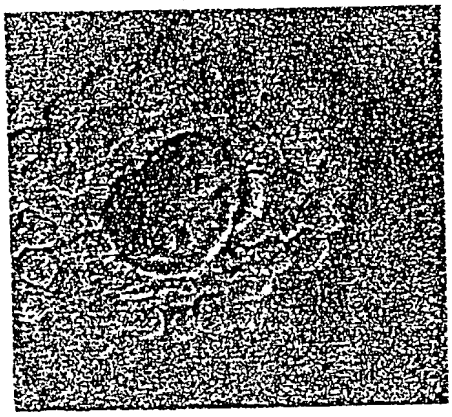


Figure 7

a



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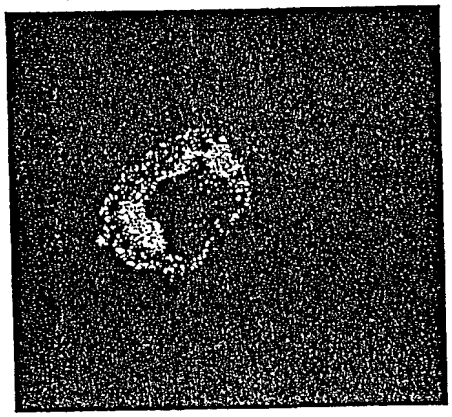

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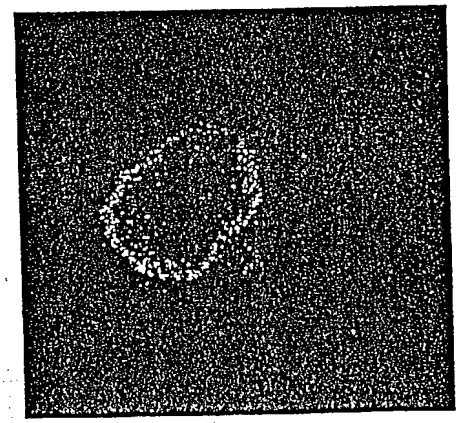

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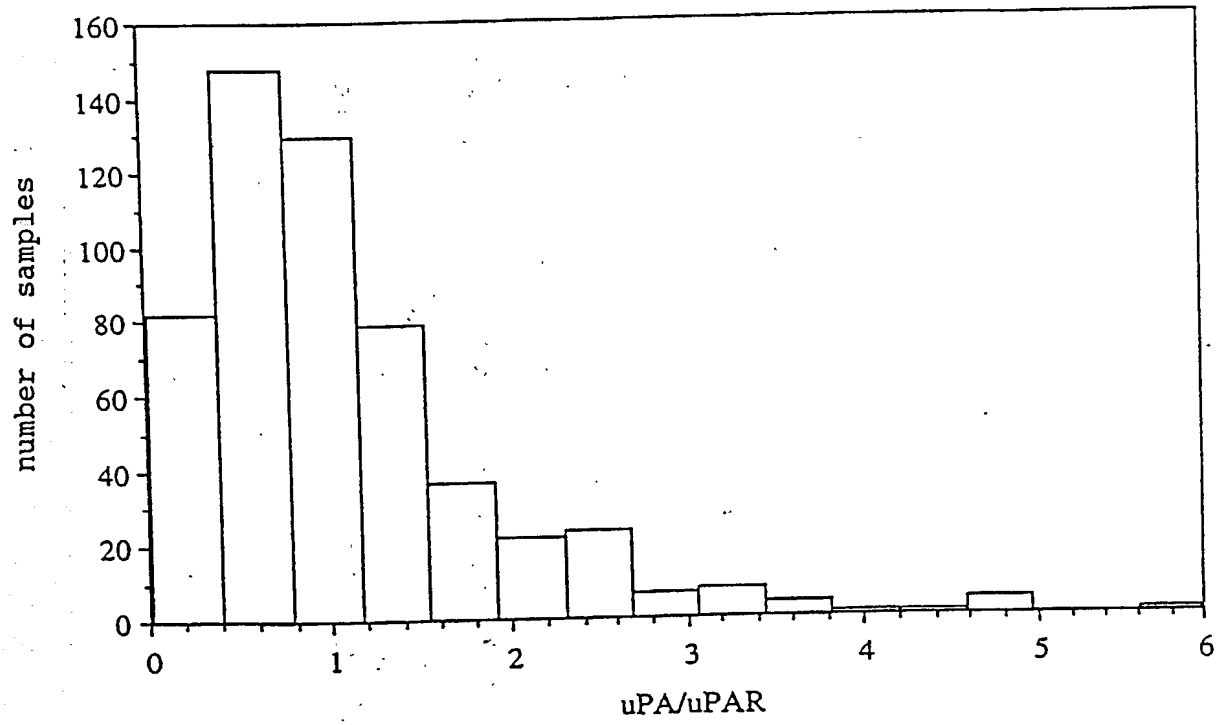
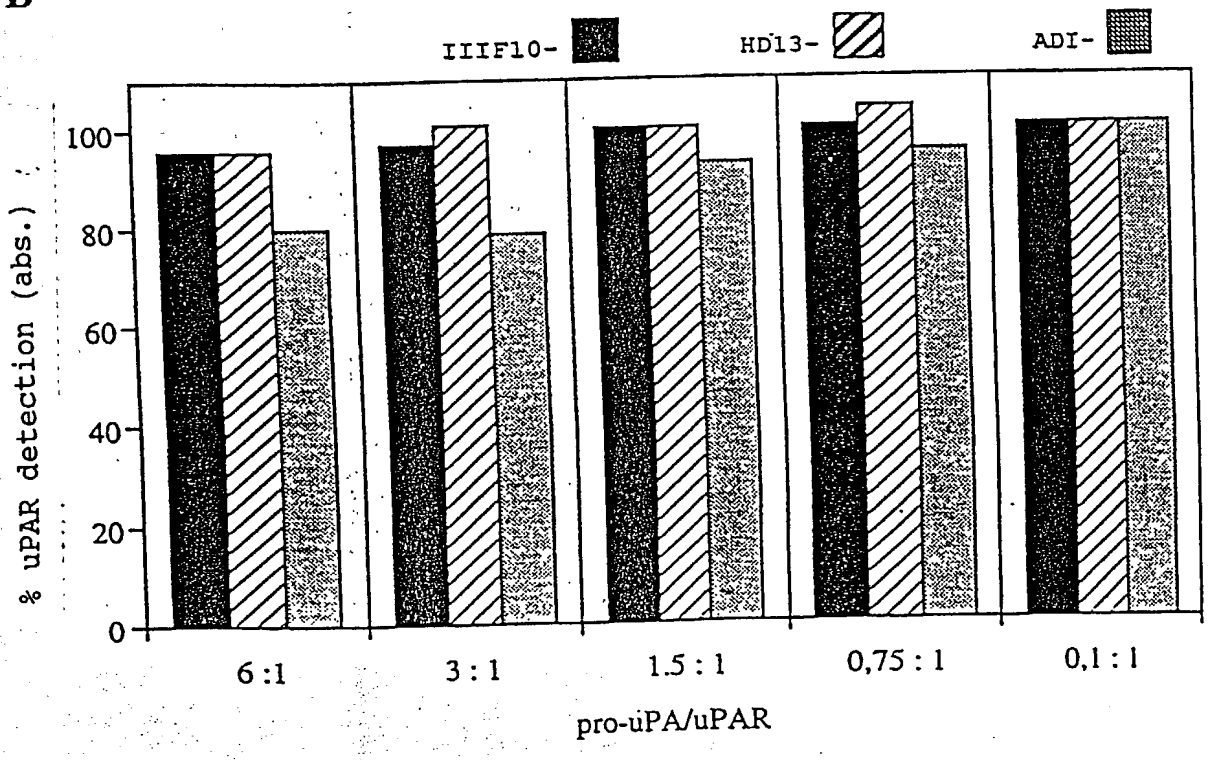
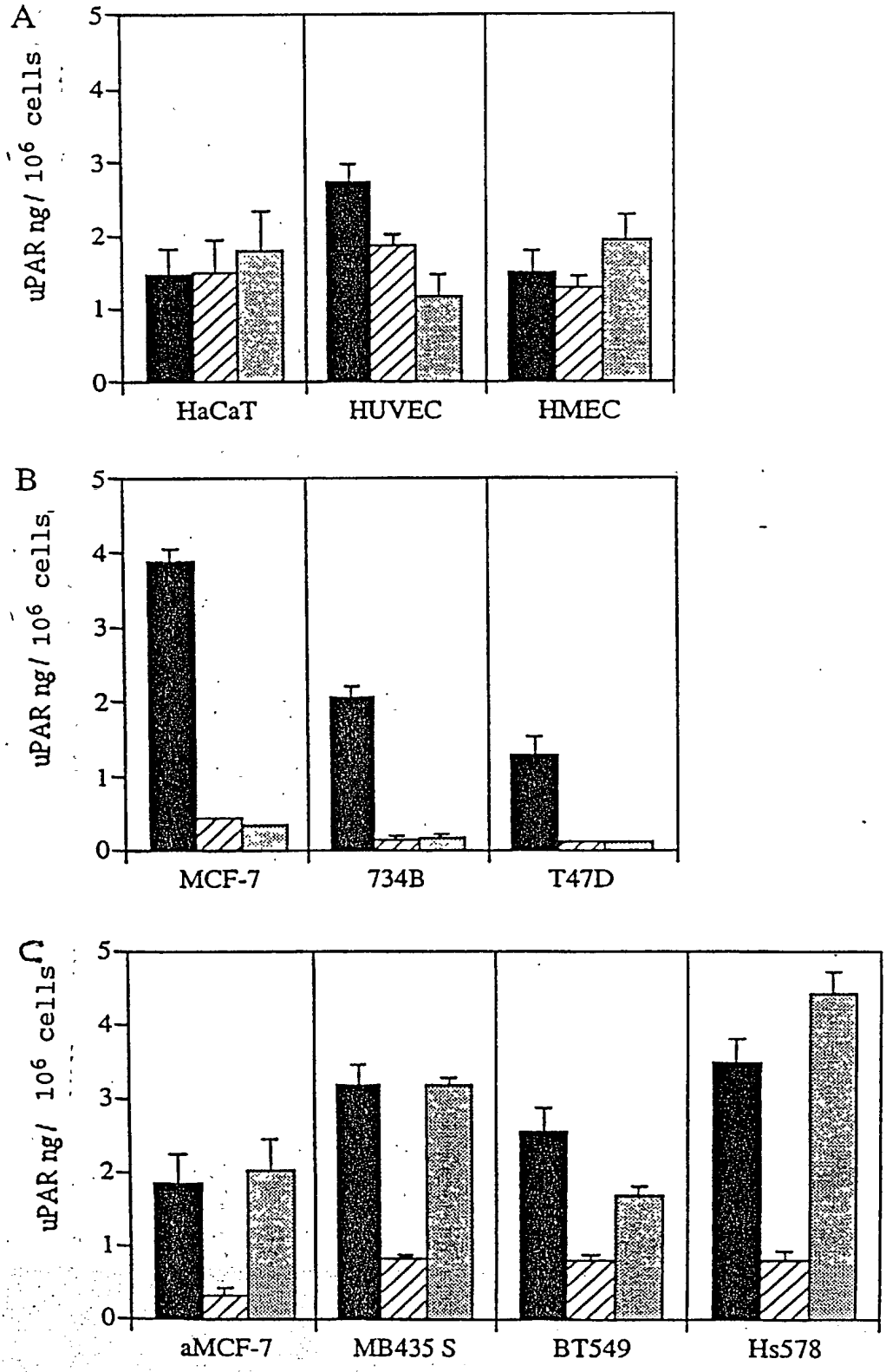

number of samples
160
140
120
100
80
60
40
20
0
0
1
2
3
4
5
6
uPA/uPAR

B


IIIF10-
HD13-
ADI-
% uPAR detection (abs.)
100
80
60
40
20
0
6:1
3:1
1.5:1
0,75:1
0,1:1
pro-uPA/uPAR


A
uPAR ng/ 10^6 cells
5
4
3
2
1
0
HaCaT
HUVEC
HMEC
B
uPAR ng/ 10^6 cells
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734B
T47D
C
uPAR ng/ 10^6 cells
aMCF-7
MB435 S
BT549
Hs578

Figure 10

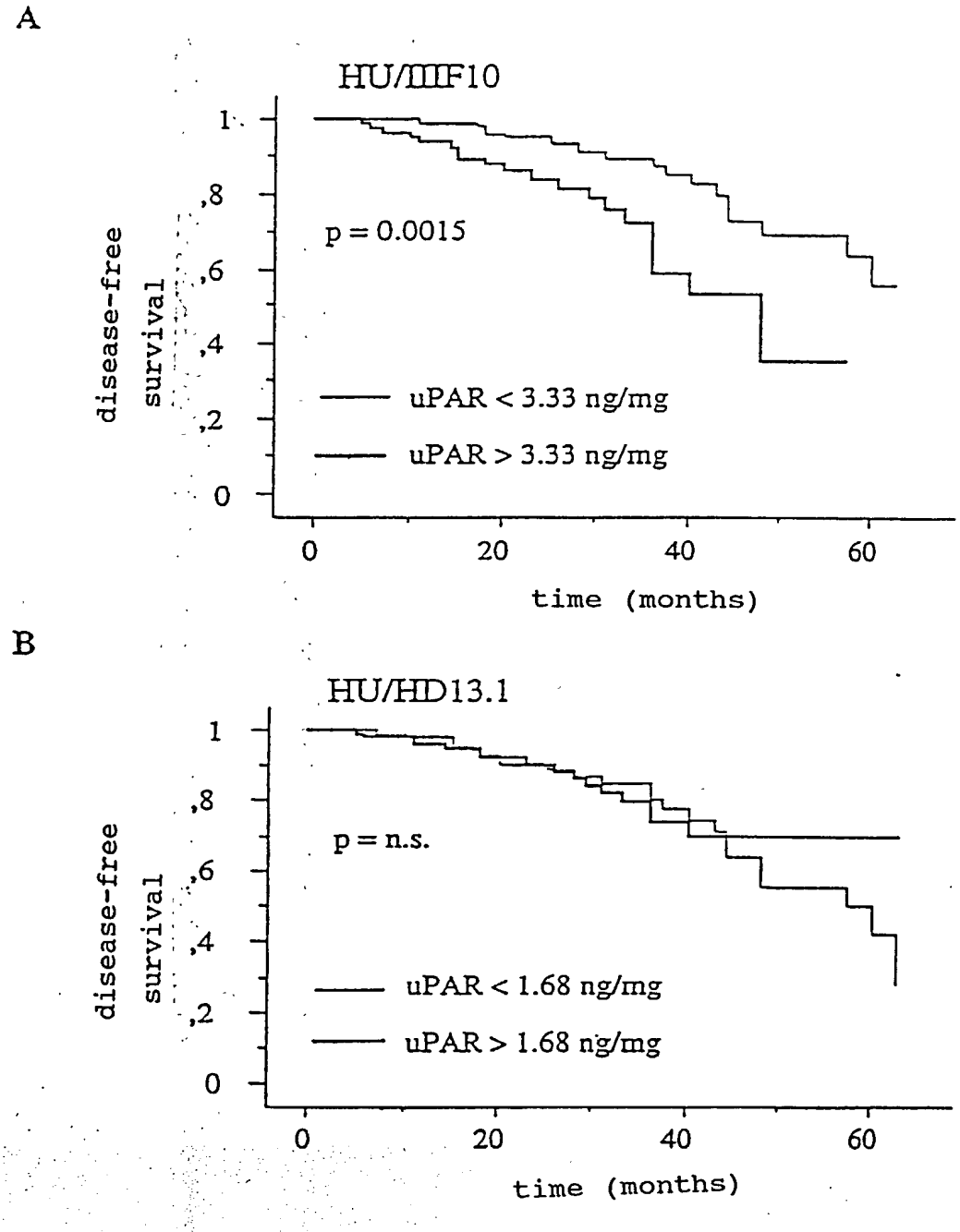

A
HU/IIIF10
disease-free survival
1
,8
,6
,4
,2
0
p = 0.0015
uPAR < 3.33 ng/mg
uPAR > 3.33 ng/mg
0
20
40
60
time (months)
B
HU/HD13.1
disease-free survival
1
,8
,6
,4
,2
0
p = n.s.
uPAR < 1.68 ng/mg
uPAR > 1.68 ng/mg
0
20
40
60
time (months)

Figure 10

C

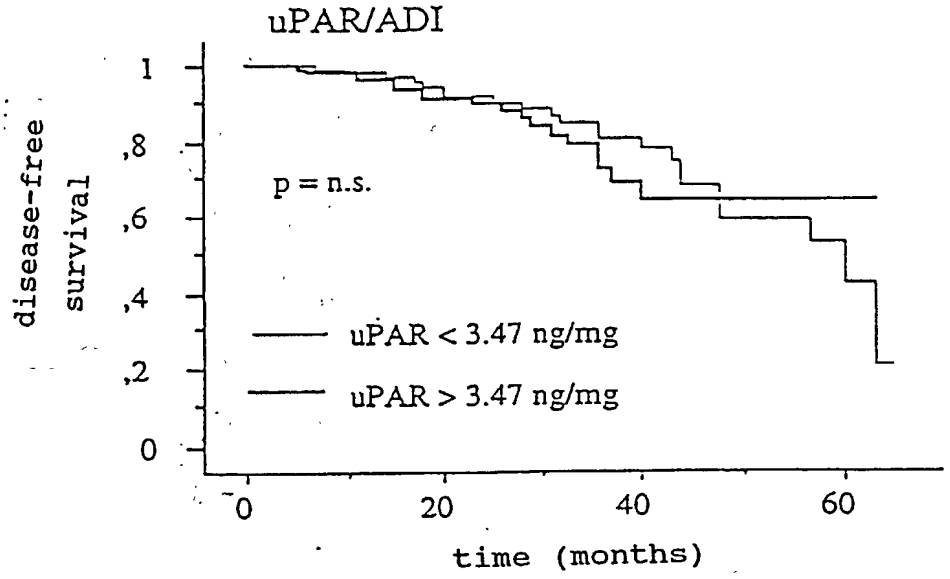

uPAR/ADI
disease-free survival
1
,8
,6
,4
,2
0
p = n.s.
uPAR < 3.47 ng/mg
uPAR > 3.47 ng/mg
0
20
40
60
time (months)

Figure 11

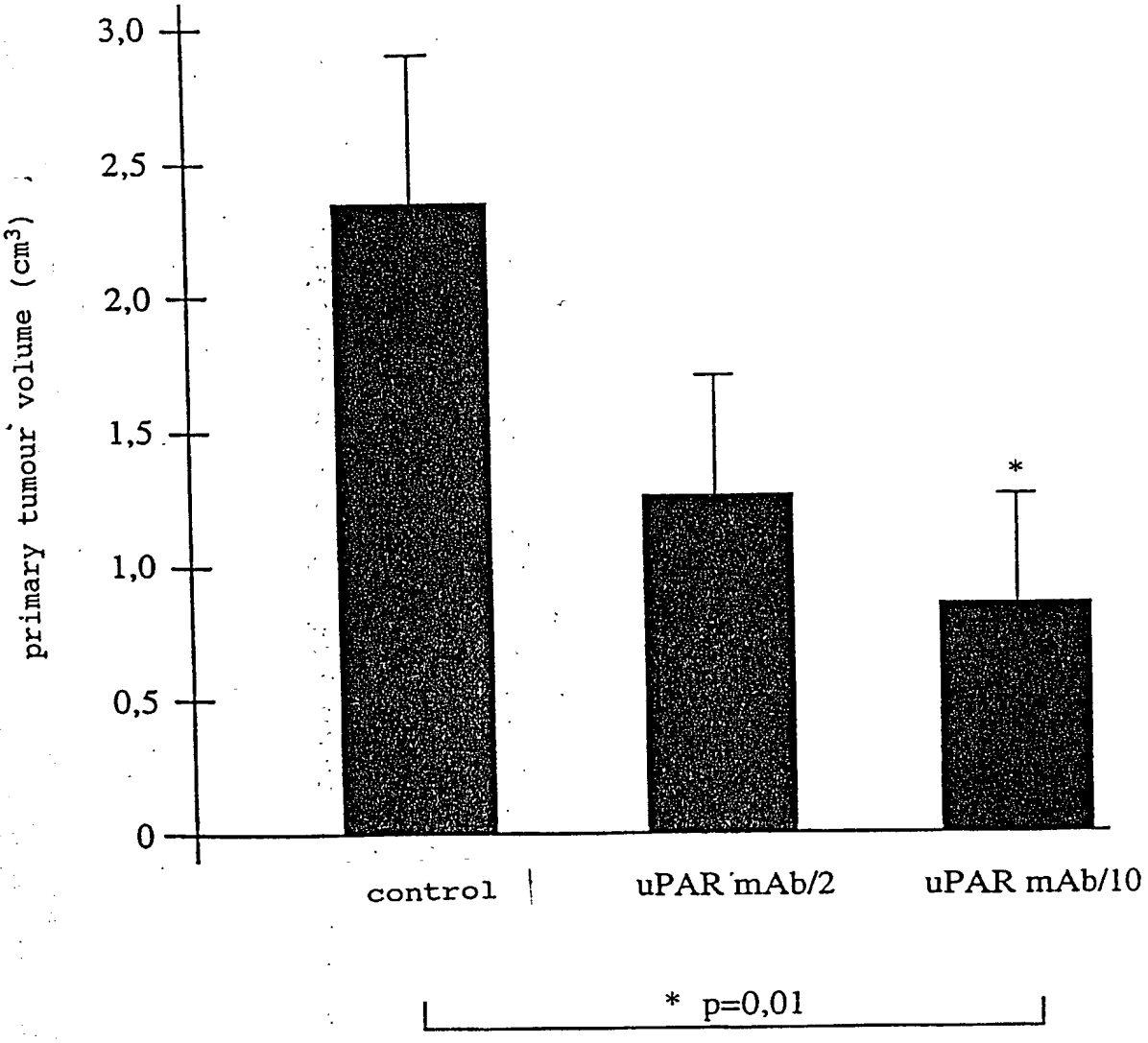

3,0
2,5
2,0
1,5
1,0
0,5
0
primary tumour volume (cm3)
*
control
uPAR mAb/2
uPAR mAb/10
* p=0,01

Figure 12

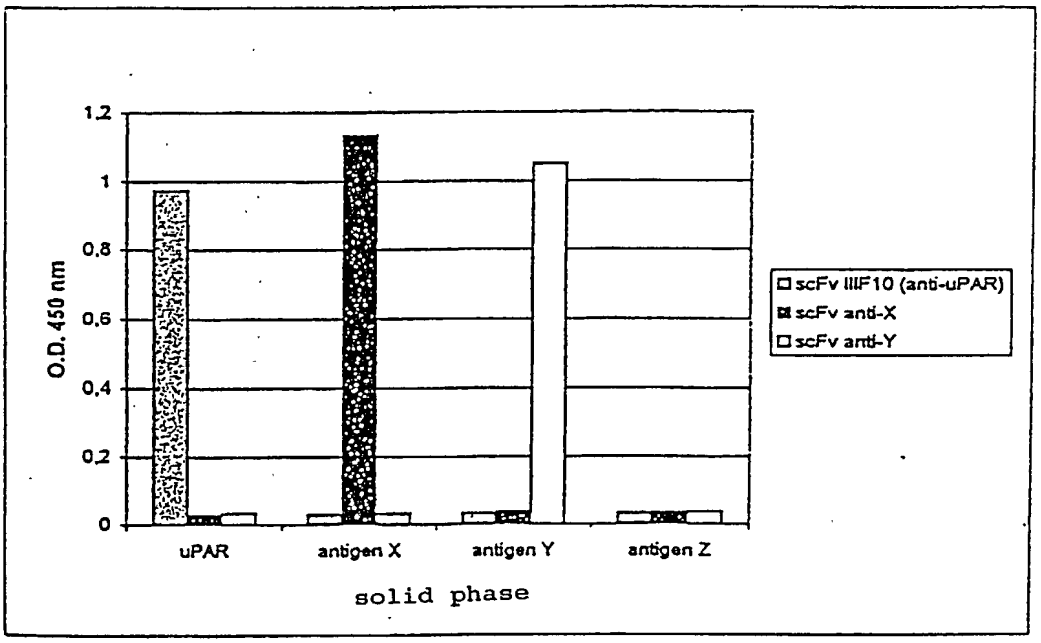
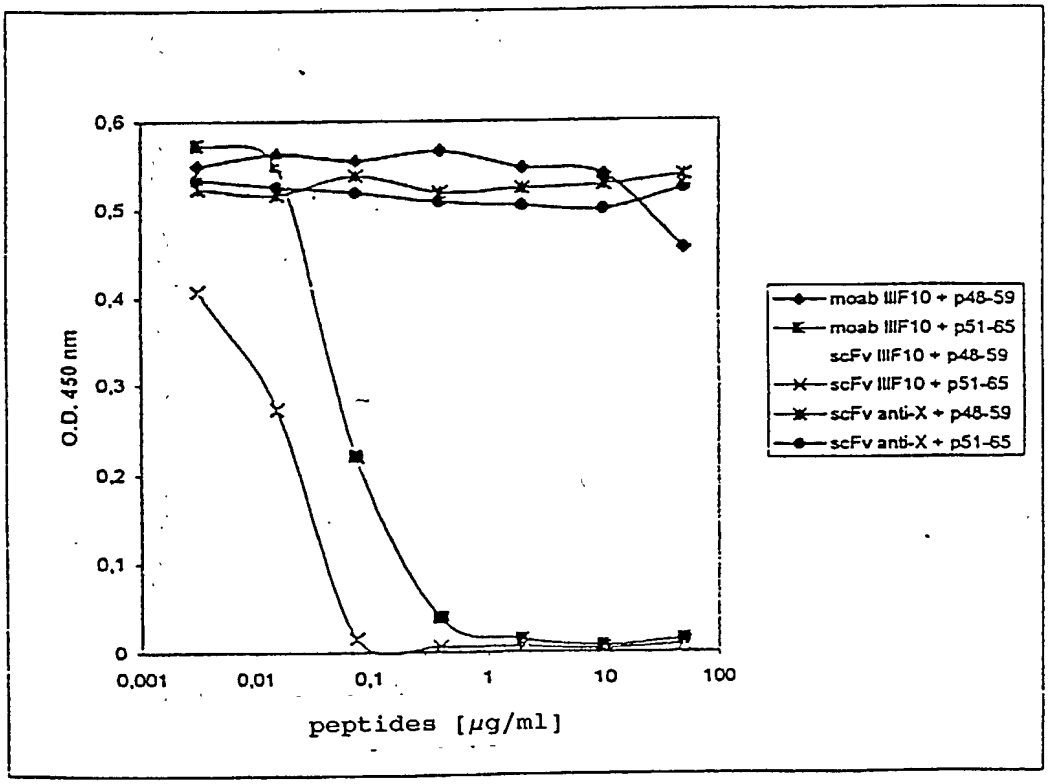

1.2
1
0.8
0.6
0.4
0.2
0
O.D. 450 nm
uPAR
antigen X
antigen Y
antigen Z
solid phase
scFv IIIF10 (anti-uPAR)
scFv anti-X
scFv anti-Y

Figure 13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0,01
0,1
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10
100
peptides [µg/ml]
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moab IIIF10 + p51-65
scFv IIIF10 + p48-59
scFv IIIF10 + p51-65
scFv anti-X + p48-59
scFv anti-X + p51-65

09/926523 .020502
05 MAR 2002

09/926,523

Docket No. 100564-00082 ARENT FOX KINTNER PLOTKIN & KAHN, PLLC

Declaration For U.S. Patent Application

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled
(Insert Title) DIAGNOSTIC AND THERAPEUTIC USE OF ANTIBODIES AGAINST THE UROKINASE RECEPTOR

the specification of which is attached hereto unless the following box is checked:

☒ was filed on April 13,2000 As PCT International Application
Number PCT/EP00/03347 and was amended on
and/or was filed on October 15, 2001 As U.S. Patent Application
Number and was amended on

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above.
I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56.
I hereby claim foreign priority benefits under 35 U.S.C. §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate or PCT International Application having a filing date before that of the application(s) for which priority is claimed:

				Priority Claimed
(List prior foreign applications)	99107199.4 (Number)	Germany (Country)	13 April 1999 (Day/Month/Year Filed)	☒ Yes ☐ No
	(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes ☐ No
	(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes ☐ No

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

(Application Number) (Filing Date)
(Application Number) (Filing Date)

☐ See attached list for additional prior foreign or provisional applications.

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or §365(c) of any PCT International application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) (U.S. or PCT) in the manner provided by the first paragraph of 35, U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(List prior U.S. Applications or PCT International applications designating the U.S.)	(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
	(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

And I hereby appoint the firm of Arent Fox, Customer Number 004372 including as principal attorneys: Robert B. Murray, Reg. No. 22,980; Charles M. Marmelstein, Reg. No. 25,895; George E. Oram, Jr., Reg. No. 27,931; Douglas H. Goldhush, Reg. No. 33,125; Richard J. Berman, Reg. No. 39,107; Murat Ozgu, Reg. No. 44,275; Robert K. Carpenter, Reg. No. 34,794; Gregory B. Kang, Reg. No. 45,273; Rustan Hill, Reg. No. 37,351; Kevin Turner, Reg. No. 43,437; Rhonda L. Barton, Reg. No. 47,271; Hans J. Crosby, Reg. No. 44,634, David D. Dzara, Reg. No. 47,543, Lynne D. Anderson, Reg. No. 46,412; Laurence J. Edson, Reg. No. 44,666, Dinnatia J. Doster, Reg. No. 45,268, Michael A. Steinberg, Reg. No. 43,160 and Lynn A. Bristol, Reg. No. 48,898.

Please direct all communications to the following address: Customer No. 004372.
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1050 Connecticut Avenue, N.W., Suite 400
Washington, D.C. 20036-5339
Telephone No. (202) 857-6000; Facsimile No. (202) 638-4810

The undersigned hereby authorizes the U.S. attorneys named herein to accept and follow instructions from the undersigned's assignee, if any, and/or, if the undersigned is not a resident of the United States, the undersigned's domestic attorney, patent attorney or patent agent, as to any action to be take in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorneys and the undersigned. In the event of a change in the person(s) from whom instructions may be taken, the U.S. attorneys named herein will be so notified by the undersigned

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1W

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2W

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Inventor's signature [signature] Date 01/14/02

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Citizenship German
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3W

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4W

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5W

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6W

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7W

Full name of sole or seventh inventor Martin MÜLLER

Inventor's signature Martin Müller X 20/02/02 Date

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Citizenship German
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8W

Full name of sole or eight inventor Olaf WILHELM

Inventor's signature X 20/2/01 Date

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Citizenship German
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9W

Full name of sole or ninth inventor Nadja HARBECK

Inventor's signature X 23/01/02 Date

Residence München (DE)
Citizenship German
Post Office Address Palnkamerstrasse 49, 83624 Otterfing, Germany

SEQUENCE PROTOCOL

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gat ata aat tgg gtg aag cgg agg cct gga cag gga ctt gag tgg att   144
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         35                  40                  45

gga tgg att ttt cct gga gat ggt agt acc aat tac aat gag aaa ttc   192
Gly Trp Ile Phe Pro Gly Asp Gly Ser Thr Asn Tyr Asn Glu Lys Phe
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aag gac aag gcc aca ctg act gct gac aaa tcc tcc agc aca gcc tac   240
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Gly Trp Ile Phe Pro Gly Asp Gly Ser Thr Asn Tyr Asn Glu Lys Phe
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             20                  25                  30

gta gcc tgg tat caa gag aaa cca ggg cag tct cct aaa gca ctg att   144
Val Ala Trp Tyr Gln Glu Lys Pro Gly Gln Ser Pro Lys Ala Leu Ile
         35                  40                  45

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Tyr Leu Ala Ser Asn Arg His Thr Gly Val Pro Asp Arg Phe Thr Gly
     50                  55                  60

agt gga tct gga aca gat ttc act ctc acc att agc aat gtg caa tct   240
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gaa gac ctg gca gat tat ttc tgt ctg caa cat tgg aat tat ccg tac   288
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Tyr Leu Ala Ser Asn Arg His Thr Gly Val Pro Asp Arg Phe Thr Gly
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Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Val Gln Ser
65                  70                  75                  80

Glu Asp Leu Ala Asp Tyr Phe Cys Leu Gln His Trp Asn Tyr Pro Tyr
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Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
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<120> Diagnostischer und therapeutischer Einsatz von
Antikörpern gegen den Urokinase-Rezeptor

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tta gtg aag ata tcc tgc aag gct tct ggt tac agt ttc aca agc tac   96
Leu Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Ser Tyr
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Asp Ile Asn Trp Val Lys Arg Arg Pro Gly Gln Gly Leu Glu Trp Ile
         35                  40                  45

gga tgg att ttt cct gga gat ggt agt acc aat tac aat gag aaa ttc   192
Gly Trp Ile Phe Pro Gly Asp Gly Ser Thr Asn Tyr Asn Glu Lys Phe
     50                  55                  60

aag gac aag gcc aca ctg act gct gac aaa tcc tcc agc aca gcc tac   240
Lys Asp Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
```

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Met Gln Leu Asn Ser Leu Thr Ser Glu Asn Ser Ala Val Tyr Phe Cys
85 90 95

gca aga gat gga agt atg ggg ggg ttt gac tac tgg ggc caa ggg acc 336
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35 40 45

Gly Trp Ile Phe Pro Gly Asp Gly Ser Thr Asn Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Asn Ser Leu Thr Ser Glu Asn Ser Ala Val Tyr Phe Cys
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Ala Arg Asp Gly Ser Met Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr
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Thr Val Thr Val Ser Ser
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Asp Val Leu Met Thr Gln Thr Pro Lys Phe Met Ser Thr Ser Val Gly
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gac agg gtc agc atc acc tgc aag gcc agt cag aat gtt cgt act act   96
Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asn Val Arg Thr Thr
             20                  25                  30

gta gcc tgg tat caa gag aaa cca ggg cag tct cct aaa gca ctg att   144
Val Ala Trp Tyr Gln Glu Lys Pro Gly Gln Ser Pro Lys Ala Leu Ile
         35                  40                  45

tac ttg gca tcc aac cgg cac act gga gtc cct gat cgc ttc aca ggc   192
Tyr Leu Ala Ser Asn Arg His Thr Gly Val Pro Asp Arg Phe Thr Gly
     50                  55                  60

agt gga tct gga aca gat ttc act ctc acc att agc aat gtg caa tct   240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Val Gln Ser
 65                  70                  75                  80

gaa gac ctg gca gat tat ttc tgt ctg caa cat tgg aat tat ccg tac   288
Glu Asp Leu Ala Asp Tyr Phe Cys Leu Gln His Trp Asn Tyr Pro Tyr
                 85                  90                  95

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35 40 45

Tyr Leu Ala Ser Asn Arg His Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Val Gln Ser
65 70 75 80

Glu Asp Leu Ala Asp Tyr Phe Cys Leu Gln His Trp Asn Tyr Pro Tyr
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
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